2017 West Point Incident Response:

Crab Tissue Sampling and Analysis Plan

Final

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1.0. INTRODUCTION

This sampling and analysis plan (SAP) presents project information and sampling and analytical methodologies for crab tissue sampling supporting the West Point Treatment Plant's incident response. King County is conducting this study to determine if wastewater discharges following the February 9, 2017 flooding of the West Point Treatment Plant, and subsequent period of reduced treatment capacity of the plant, adversely influenced crab tissues. This will be done by determining if there are substantive increases in toxicant concentrations in Dungeness crab tissue following these events when compared to historical crab tissue data collected by either King County (King County 2016) or Washington Department of Fish and Wildlife (WDFW) (Carey et al 2014). This collection effort is a collaborative effort between King County and WDFW.

1.1 Project Background

King County's West Point Wastewater Treatment Plant is located near the west side of Magnolia Bluff, adjacent to Seattle's Discovery Park (Figure 1). The regional treatment plant serves a combined system that receives both wastewater and stormwater. The plant began operation in 1966 providing primary wastewater treatment, and was upgraded to secondary treatment in 1995. The average annual secondary treatment volume of the plant is 95 million gallons per day (mgd), with an average wet-weather flow of 133 mgd and a peak wet-weather influent capacity of 440 mgd. Secondary treatment at the West Point plant consists of screening, grit removal, primary sedimentation, air-activated sludge, secondary sedimentation, disinfection by chlorination, and anaerobic digestion of solids. Secondary effluent is dechlorinated prior to discharge.

Treated effluent is discharged to Puget Sound through a marine outfall at a discharge point approximately 3,600 feet (914 m) offshore to the west of West Point at a depth of -240 feet (-73 m) referenced to mean lower low water (MLLW). The outfall pipeline is buried for much of its extent, daylighting shortly before the start of the diffuser. The diffuser, located at the end of the outfall, is approximately 610 feet (186 m) in length. Bathymetry around the outfall diffuser is relatively flat, with depths ranging from approximately -210 to -250 feet MLLW (-64 to -76 m). In addition to the primary outfall, the plant has an emergency bypass outfall (EB0) which is located 500 feet offshore on the north side of West Point near Discovery Park (Figure 1). The discharge point is at an approximate water depth of -35 feet (-11 m) MLLW.

On February 9th, 2017 an emergency bypass event occurred due to equipment failure during peak inflows at the West Point Treatment Plant. This bypass resulted in the release of untreated wastewater and stormwater into Puget Sound through the EBO. Two smaller untreated discharge events via the EBO occurred on February 15 and 16. In total, about 235 million gallons of untreated stormwater and wastewater were discharged. No untreated discharges have occurred through the EBO following the event on February 16 and effluent has subsequently been discharged through the main outfall.

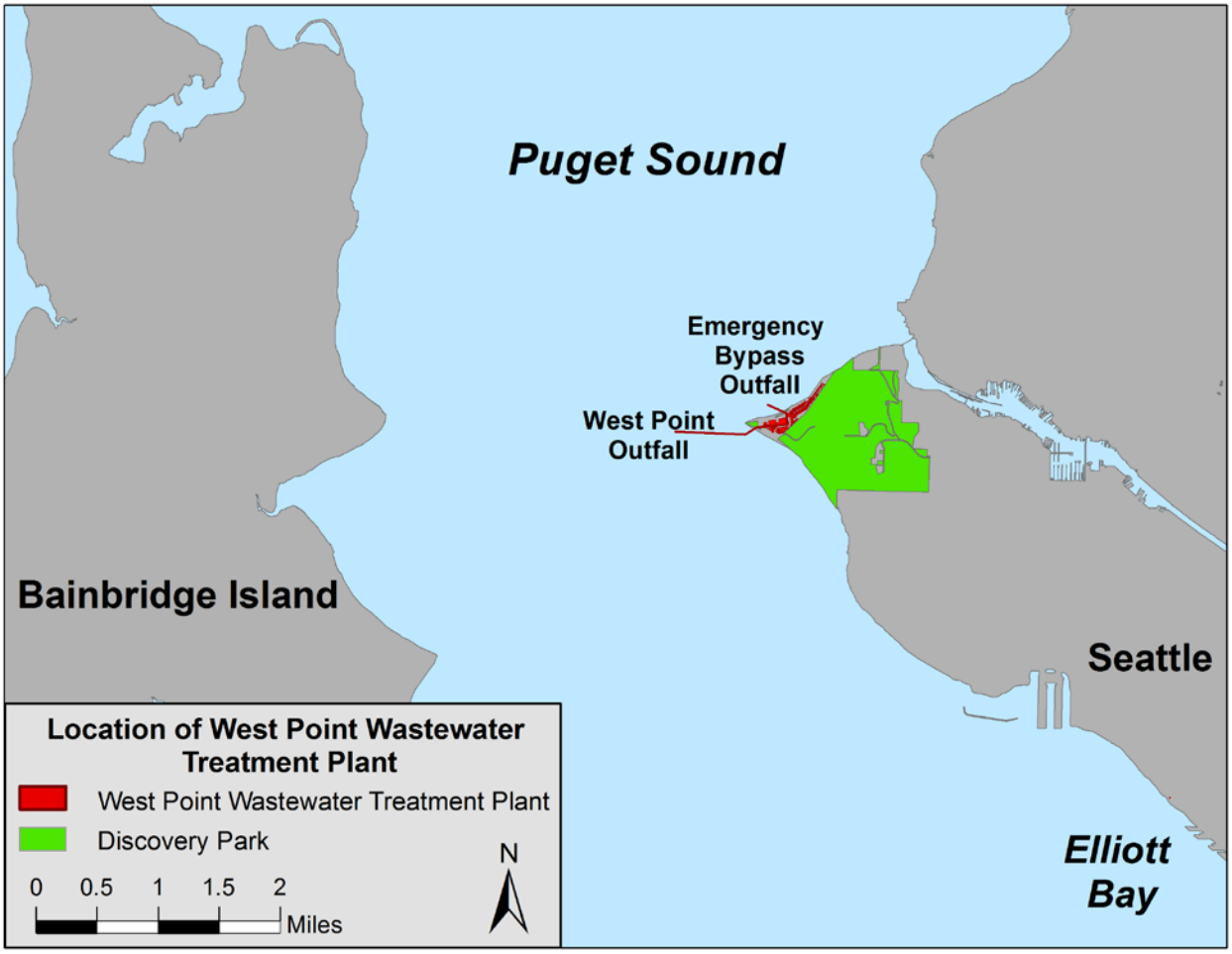

Figure 1. Location of West Point Wastewater Treatment Plant and associated outfalls.

After the February 9 discharge event, the plant operated using limited primary treatment. This includes some settling of solids, screening, disinfection, and dechlorination of wastewater. Efforts to restore secondary treatment processes occurred from the event date until April 27, but not all wastewater was receiving full secondary treatment during this period. Between April 27 and May 9, all wastewater was receiving full secondary treatment; however, solids handling processes were still in a period of adjustment that affected the ability of the plant to routinely meet the water quality effluent limitations. In total, approximately 10.7 billion gallons of effluent that exceeded some of the permit requirements for water quality were discharged into Puget Sound through West Point's deep-water outfall. On May 10 the treatment plant began meeting all National Pollutant Discharge Elimination System (NPDES) permit limits.

1.2 Incident Response Monitoring

In addition to repairing the West Point Treatment Plant, King County increased monitoring efforts in Puget Sound. The initial response was to increase monitoring of Puget Sound waters to assess if the effluent during the period of temporarily reduced treatment resulted

in measurable effects to water quality. The response included increasing the frequency of ongoing offshore monitoring efforts, adding a monitoring station at the EBO, increasing bacteria monitoring at beaches, expanding nutrient monitoring, and measuring trace metals concentrations in the water column.

In addition to measuring potential effects in the water column, King County has been working to identify effects to marine sediments and tissues near the West Point treatment plant. Related monitoring efforts include:

- Sediment conventionals (e.g., total organic carbon, sulfides, particle size distribution, etc.), trace metals, organic compounds, and benthic invertebrates near the deep outfall (King County, 2017a,b)
- Metals and organic compounds in tissues of English Sole (with WDFW) and zooplankton (King County 2017c)
- Metals in clam tissue and intertidal sediments (King County 2017d)

The objective of this SAP is to evaluate if discharges during the incident were associated with an increase in crab tissue contaminant levels by collecting Dungeness crab and analyzing tissues for metals and organic compounds. The data will be compared to existing Dungeness crab data collected from same area as part of King County's Tissue Monitoring Program (King County 2015), as well as previous data collected by WDFW in nearby areas of Puget Sound.

1.3 Scope of Work

This monitoring will include collection of Dungeness (*Metacarcinus magister*) crab from two locations near Shilshole Marina. Crab muscle and hepatopancreas tissue composite samples will be analyzed for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), metals and mercury, as well as conventional parameters. Crab muscle tissue will also be analyzed for carbon and nitrogen stable isotopes. Stable isotopes are commonly employed in toxicity and food web studies to investigate trophic dynamics. Stable nitrogen isotopic signatures supply information about the trophic position and diet of consumers (Peterson and Fry 1987; Fry 1988; Peterson et al. 1985), while stable carbon isotopic signatures are useful for distinguishing among food webs based on different types of primary producers or the location in which consumers feed along a salinity gradient (e.g., Stewart et al. 2004; France 1995).

2.0. PROJECT ORGANIZATION AND SCHEDULE

The tasks involved in conducting the 2017 West Point incident response crab tissue monitoring event and the personnel responsible for those tasks are shown below.

- **Debra Williston** – King County Toxicology and Contaminant Assessment Unit – 206-477-4850 – debra.williston@kingcounty.gov – Project management, study design, preparation of SAP, management of data validation and analysis, and preparation of study report.
- **Rory O'Rourke** – King County Toxicology and Contaminant Assessment Unit – 206-477-4715 – rory.orourke@kingcounty.gov – Preparation of SAP.
- **Deb Lester** – King County Toxicology and Contaminant Assessment Unit – 206-477-4752 – deborah.lester@kingcounty.gov – Study design, review of SAP and study report.
- **Jeff Lafer** – King County Wastewater Treatment Division – 206-477-6315 – jeff.lafer@kingcounty.gov – Study Design approval and review of SAP and study report.
- **Bob Kruger** – King County Environmental Laboratory – 206-477-7147 – bob.kruger@kingcounty.gov – Lead of field sampling activities.
- **Fritz Grothkopp** – King County Environmental Laboratory – 206-477-7114 – fritz.grothkopp@kingcounty.gov –King County Environmental Laboratory project management, coordination of all laboratory activities and data reporting.
- **Colin Elliott** – King County Environmental Laboratory – 206-477-7113 – colin.elliott@kingcounty.gov – Internal review of SAP, coordination of King County Environmental Laboratory QA/QC programs.

Crab will be collected in May and September (or October) 2017 over a 3-day period. The King County Environmental Laboratory (KCEL) will analyze all crab tissue samples. The turn-around time for analytical data is approximately eight to ten weeks from the date of last sample receipt, at which time entry of quality assurance (QA)-approved data into the laboratory information management system (LIMS) should be complete. A report will be prepared after all analytical data has been analyzed and a QA review completed. The report is anticipated to be completed by first quarter 2018.

3.0. SAMPLING DESIGN

The following sections provide a description of the study design including data quality objectives (DQOs), sampling methods, and field and analytical parameters.

3.1 Data Quality Objectives

The project manager will assess project data and evaluate whether the data collected are of sufficient quality to meet monitoring goals. The DQOs of precision, accuracy, bias, representativeness, completeness, comparability, and sensitivity are described in the following sections, along with data assessment. Data not meeting objectives will be identified and potentially used to improve future monitoring efforts.

3.1.1 Precision, Accuracy, and Bias

Precision is the agreement of a set of results among themselves and is a measure of the ability to reproduce a result. Accuracy is an estimate of the difference between the true value and the measured value. The accuracy of a result is affected by both systematic and random errors. Bias is a measure of the difference, due to a systematic factor, between an analytical result and the true value of an analyte. Precision, accuracy, and bias for analytical chemistry may be measured by one or more analyses of various laboratory QC samples such as blanks, surrogates, and replicates.

Field collected tissue samples are expected to be fairly variable between samples and mostly driven by size and species. Laboratory precision will only be evaluated by the use of laboratory quality control samples, including spikes and spike duplicates.

Analytical bias cannot be quantified for tissue analysis, particularly for PCB homologs, which are mixtures of different PCB congeners. However, the composite crab samples will target average concentration and reduce some of the natural variability between individual crab.

Standard Reference Materials (SRMs) will be used whenever available to better quantify specific analytical method accuracy and precision.

3.1.2 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point, or an environmental condition. The species and size of crab targeted for collection were selected so general comparisons to past collection efforts can be made. Following the guidelines described in Section 4 for sample collection, processing, and handling will also help ensure that samples are representative.

3.1.3 Completeness

Completeness is defined as the total number of samples analyzed for which acceptable analytical data are generated, compared to the total number of samples submitted for analysis. Sampling with adherence to standardized sampling and testing protocols will aid in providing a complete set of data for this survey. The goal for completeness is 100%. If 100% completeness is not achieved, the project team will evaluate if the DQOs can still be met or if additional sample collection and analysis is necessary prior to data reporting and analysis.

3.1.4 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal is achieved through using standard techniques to collect and analyze representative samples, along with standardized data assessment and reporting procedures. By following the guidance of this SAP, the goal of comparability between this, past and future sampling events will be achieved. Use of established techniques enhances regional comparability.

3.1.5 Sensitivity

Sensitivity is a measure of the capability of analytical methods to meet the monitoring goals. The analytical method detection limits presented in Section 5 are sensitive enough to detect analytes at concentrations of interest to assess significant changes in crab tissues.

3.1.6 Data Assessment

Chemical data and quality control (QC) results will be assessed against requirements of the analytical methods as well as the requirements of this SAP.

3.2 Sampling and Analytical Strategy

Crab will be collected using pots deployed by boat near Shilshole Bay Marina. The crab pots will be deployed overnight for collection at two sampling locations. Two sampling events (May and September) will occur. Target sample numbers are listed in Table 1.

Table 1. Target number of Dungeness Crab by sampling event

Sample Event	Tissue type	Target number of composites	Target number of crab per composite	Total Crab
May	Muscle	5	3	Up to 30
	Hepatopancreas	3	5	
September	Muscle	5	3	Up to 30
	Hepatopancreas	3	5	

Note: The number of crab per composite will be consistent across all samples; the final number will be based on the number of crab collected.

3.2.1 Sampling Station Locations and Sample Identification

Crab will be collected from a north and south location off-shore from Shilshole Bay Marina breakwater (see Figure 2). This location is similar to the previous collection effort by King County in 2014. WDFW suggested that the locations off-shore of the breakwater are better habitat for Dungeness crab than the location within the breakwater closer to the fishing piers.[1]

A Locator Identification code generated for each of the two sampling locations is shown in Table 2. Unlike other types of sampling, the exact location of crab pot deployment does not need to be precise to ensure sampling consistency. However, at the same time, it is helpful to have a record of the general location where crabs were collected. Therefore, KCEL Field Science Unit (FSU) staff will report the coordinates of each crab pot per deployment and note how many of each of the targeted species were retained from each pot deployment. This information will be recorded on field sheets (see Appendix A and Section 4.1 for additional field sheet recordings). This information will also be recorded in the LIMS. The locator coordinates used for these samples will be a point on the associated fishing pier.

Table 2. Locator ID and general coordinates for each sampling location

Location	Locator ID	Easting	Northing
Shilshole Bay Marina North	CB-SHMarina-N	1253476	254630
Shilshole Bay Marina South	CB-SHMarina-S	1251797	250856

[a] Coordinates represent general sampling locations. Coordinates are in State Plane North NAD83 US Survey Feet.

[1] Attempts to collect Dungeness crab from inside the breakwater were first attempted in May 2017 but only red rock crabs were collected. The location was moved to off-shore of the breakwater on advice from WDFW.

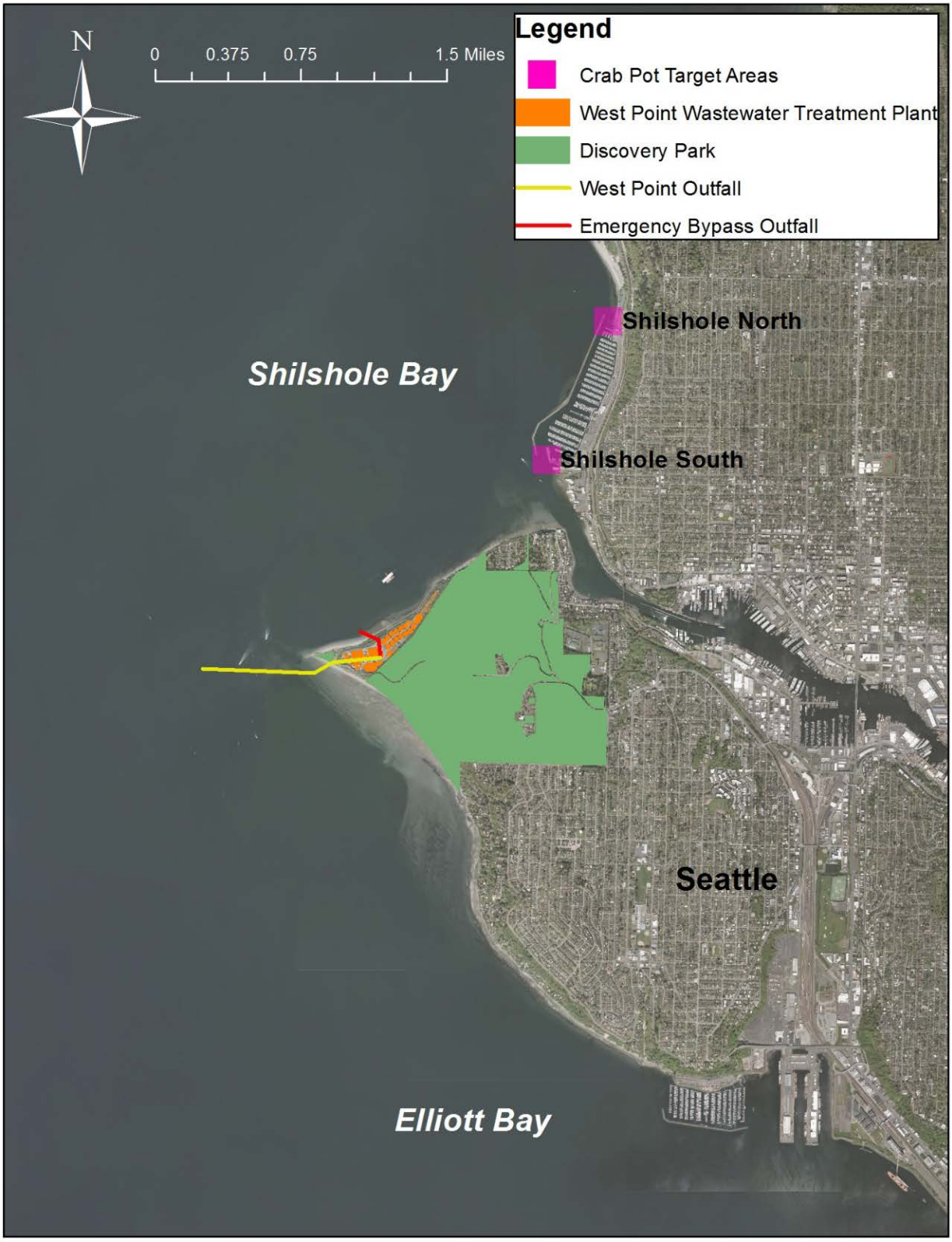

Figure 2. Crab sampling locations

3.2.2 Sample Acquisition and Analytical Parameters

Sampling will be conducted using commercially available recreational crab pots. Sampling will be conducted by KCEL FSU staff. Samples will be collected under the authorization memos issued by WDFW.

Each sample will be analyzed for total metals (arsenic, beryllium, cadmium, chromium, copper, lead, nickel, selenium, silver, thallium, and zinc), mercury, PCB homologs, PBDEs, PAHs, stable isotopes, total solids, and lipids. All chemical and conventional analyses will be conducted by KCEL except stable isotopes. If constraints, such as sample mass, limit analyses, the following prioritization will be followed:

1. PCB homologs
2. Total Solids
3. Total metals
4. Total mercury
5. Total lipids
6. PBDEs
7. PAHs
8. Stable isotopes

The minimum tissue mass required for each analysis type is presented in Table 3.

Table 3. Minimum analytical mass requirements for tissue samples by matrix

Analyte	Muscle Tissue / Heptopancreas	Extra mass for Quality Control Analyses
PCB Homologs	20 g / 10 g	80 g for muscle; 40 g for hepatopancreas
Metals	1.25 g / 1.25 g	2.5 g for muscle; 2.5 g for hepatopancreas
Mercury	1 g / 1 g	2 g for muscle; 2 g for hepatopancreas
PBDEs	20 g / 10 g	80 g for muscle; 40 g for hepatopancreas
PAHs	20 g / 10 g	80 g for muscle; 40 g for hepatopancreas
Stable Isotopes	1 g / NA	5 g for muscle
Total Solids	1 g / 1 g	1 g for muscle; 1 g for hepatopancreas
Lipids	NA	Co-extracted with PCB Homologs
Total Mass needed	**64.25 g / 33.25 g**	**250.5 g for muscle; 125.5 g for hepatopancreas**

4.0. SAMPLING PROCEDURES

This section describes the sampling procedures to be followed over the course of all sampling events to meet the monitoring DQOs: representativeness, comparability, and completeness. Sample handling, storage, and preparation will generally follow EPA (2000) guidance.

4.1 Sample Collection

Pending development of this SAP, a technical memorandum outlining crab collection and sampling procedures was developed for the May sampling event (King County 2017e- see Appendix B).[2] This was necessary because the timing of the first sampling event did not allow time for a complete SAP. This SAP will be used to implement the September sampling event.

The field lead will ensure that a copy of the collection authorization memo obtained from WDFW is with the sampling crew and crab pots are appropriately labeled. Crab will be collected using large recreational crab pots deployed from a boat. Crab pots will be baited with fish (either heads/tails or herring), squid, cockles and/or poultry necks/legs. Crab bait will be placed in mesh bait bags or boxes and tied to the inside of the trap so the bag cannot be opened and its contents consumed. Two crab pots will be deployed at each location for approximately 12-20 hrs (overnight) and then retrieved. A target of 15 male Dungeness crabs will be collected at each of the two locations. Sampling will be conducted over a 3-day period. If 15 crabs are collected at a location within a shorter period, no further collections will occur at that location and these additional pots maybe deployed at the other location to increase the probability of collecting the target number of crabs.

Legal sized crab will be targeted, thus carapace size should be at least 6.25" for male Dungeness. However if only undersized crab are being caught, crab within ½ inch of legal size will be retained. Crab carapace width measurements will be made in the field using crab gauge (available at marine supply stores) to ensure appropriate size is being retained. In keeping with WDFW guidance, crab carapace width measurements will be made laterally across the carapace from just inside points. Only male Dungeness will be retained. Otherwise excess or undersized crab, softshell crab, or female Dungeness crab will be returned to the water immediately at their location of capture. The types and number of species caught in the crab pots will be recorded on field record sheets for each location (see Appendix A). The size range will be estimated for those crabs under the legal size. For those crab retained for analysis, the field sheets will record the species, number, collection date, pot number and pot coordinates.

The pot number will be a simple letter and number sequence. Pots will be labeled A, B, C, D, etc., and then simple number sequence added for each pot deployment. For example, at Shilshole North, the first pot deployed will be Pot A-1, the second pot deployed will be Pot

[2] This memo uses sampling methods outlined in previous crab SAP (King County 2014), which is consistent with sampling methods described in this SAP.

B-1. If these pots are deployed on another day at this location, they would be pot A-2 and B-2. If a pot from one location is deployed at another location, the letter designation will remain and the pot number sequence will be the next one available. For example, a pot that had been used twice at Shilshole North is used at Shilshole South, the pot would be labeled A-3 (this states this is the 3rd deployment for this particular pot). Because the locator and pot coordinates are on the collection form, the location the pot is deployed will be known.

Field staff will wear protective thick vinyl or plastic gloves for personal protection from crab claws. Also, because the target tissue for this study is muscle and hepatopancreas, the shell and shell membranes of undamaged specimens afford protection from potential environmental contaminants during the retrieval from the pot. For these reasons, field staff will not be required to wear nitrile gloves. To optimize the natural shell and membrane protection, specimens with damaged shells will not be accepted as samples.

After crab traps have been retrieved, captured crabs are photographed. Target crab specimens will be measured with crab gauge in the field to ensure target size is retained; detailed measurements of size and weight of retained crab will be conducted at KCEL. Acceptable crabs are bagged and placed on ice in coolers until they can be frozen, which will be no more than 12 hours after collection. Individual specimens from a particular pot will be kept together in one large resealable plastic bag(s) with the date, time, locator, pot number, and species recorded on the outside in indelible ink. All other pertinent information will be traceable through the field sheets or field notebook. The bagged and iced crabs will be transported in coolers to KCEL on the same day they were collected.

4.2 Field Sampling Equipment

The items needed in the field for each sampling method are identified below. The field lead will check that all equipment is included and in working order each day before sampling personnel go in the field.

1) Crab Pots
    a) Pot with lines, floats, weights
    b) Bait
    c) Crab Pot labels with King County name and address
2) Sampling supplies:
    a) Coolers
    b) Ice
    c) Gallon size and 2-gallon size freezer plastic bags
    d) Pens and markers with indelible ink
    e) Crab gauge
    f) GPS
    g) Nitrile gloves (optional)

3) Safety equipment:
    a) Personal floatation device
    b) Thick vinyl or plastic gloves for personal protection
    c) Rain gear
    d) Cellular phone
    e) First aid kit
4) Documentation supplies:
    a) Field notebook and/or field sheets (with Chain-of-Custody stamp)
    b) Sample labels
    c) Camera

4.3 Sample Processing

The following describes the steps for sample processing and homogenizing of crab samples. Dissection and homogenization procedures follow the recommendations in EPA (2000). Homogenization of composite samples will not occur until project manager has been consulted on the final compositing scheme, which will depend on the numbers and species of crab collected.

At the end of each sampling day, the iced crabs will be brought to KCEL for initial processing. Immediately upon return from the field, weighing and measuring of the crab will occur. The weight, carapace width, species, and sex will be recorded on the processing sheet (see Appendix C). Crab carapace width measurements will be obtained using stainless-steel calipers. Crabs will be weighed using a laboratory balance suited for the weight of the species. Crab will be identified to species, measured to the nearest 1 mm, and weighed to nearest 0.5 g. This information will be recorded on sample processing sheet (see Appendix C). Crabs unique to a location and pot deployment will be recorded on individual processing sheets. In keeping with WFDW guidance, crab carapace width measurements will be made laterally across the carapace from just inside points (Figure 3). Final processing steps, which include dissection of muscle meat and hepatopancreas, homogenization of tissues, and packaging tissue homogenates samples for freezer storage, will occur following completion of all crab collection efforts.

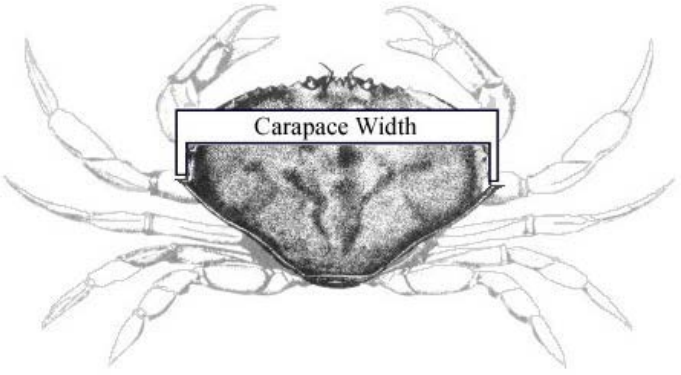

Figure 3. Location of crab carapace width measurements

In general, composite samples will comprise individual crabs of different carapace size such that average size is similar between composite samples. Composites will be composed of three crabs per muscle tissue sample and five crabs per hepatopancreas tissue sample, with each crab supplying approximately equal mass of muscle or hepatopancreas tissue respectively. The final compositing scheme will be determined in consultation with the project manager after completion of all collection efforts. Both muscle and hepatopancreas tissues will be dissected from the crab using cleaned stainless steel implements. Muscle tissues will be homogenized with a Tissumizer®, while hepatopancreas will be homogenized in the sample jars using a spatula. Each composite should be comprised of approximately 150-250 g of muscle tissue and 125 g of hepatopancreas tissue. As noted in Table 3, the minimum amount is 33 g of muscle tissue and 23 g of hepatopancreas but for samples targeted for QA analyses at least 400 g of muscle and 100 g of hepatopancreas tissues are needed. Sample jars will be labeled and placed in freezer storage (see Section 4.4). Composited crab will be recorded on the Sample Compositing Sheet (see Appendix C). This sheet will include recording of mass aliquot including in the composite sample along with information from the sampling processing sheet in order to track the size and species of crab tissue per composite.

Final processing of samples will follow these general steps:

1) Frozen crab will be thawed enough to allow processing.
2) Knives and sample processing tools (forks, hemostats, pliers) will be detergent washed, DI rinsed, propanol rinsed, and air dried. Knives and other instruments may be ceramic, stainless steel, or PTFE (Teflon). The same clean instruments/surface can be used repeatedly, without re-cleaning, on specimens contributing to the same composite; however, different pre-cleaned tools will be used between hepatopancreas and muscle tissues. All processing equipment must be subjected to the complete cleaning procedure between composites. Lab personnel will wear nitrile gloves that must be changed between composite samples. A “clean” work-surface, means a surface (lab counter, cutting board, sorting tray, etc.) covered by aluminum foil fresh off the roll. The work surface is

covered with at least one layer of aluminum foil and the foil must be changed between composites.

3) Working on a cleaned polypropylene (PP) cutting board, which can be covered with aluminum foil, the abdomen of each crab will be removed to access the hepatopancreas which will be removed first and placed directly into a labeled sample jar. The hepatopancreas also known as "crab butter" is observable as a greenish to brownish paired organ. It can be removed while the crab is still slightly frozen or thawed; avoiding significant mixing of body fluids, or loss of tissue if it becomes fluid.
4) The remainder of the crab body will be dissected using a knife and forceps while the claws will be separated from the body. Muscle tissue for the composite should be taken from the body, legs and the claws. A hemostat or tweezers may be used to remove muscle from the claws or cleaned pliers may be used to break them open. Once removed, muscle tissue will be placed into labeled sample jar.
5) Individual crab muscle tissue will be homogenized in sample jar with food grinder. Individual crab hepatopancreas tissue will be homogenized in sample jar with a spatula.
6) After all crab in the composite sample are dissected and tissue homogenized, an equal mass aliquot of muscle tissue from each crab will be transferred to a new sample jar and homogenized with a Tissumizer. This step is repeated in separate container for hepatopancreas tissue using a spatula.
7) Wash and dry PP cutting board, dissection tools, and the blender between composite samples.
8) Completed homogenized samples will be stored frozen until analysis (see Section 4.4). Prior to analysis, tissue will be thawed and sufficient mass (either muscle or hepatopancreas) from the jar will be removed for analysis; extra homogenate tissue will be archived.

4.4 Sample Delivery and Storage

Crab will be held in labeled plastic bags on ice in the field. At the end of each sampling day, all crabs will be transported back to the KCEL, initially processed as described in Section 4.3 and then stored frozen at KCEL until final processing. Final processing of crab will occur within approximately 10 days of last collection day. After all processing complete, the holding times in Table 4 apply to the composite samples.

Table 4. Sample containers, storage conditions, and analytical hold times

Analyte	Container	Preferred Storage Conditions	Hold Time	Acceptable Storage Conditions	Hold Time
Total Solids	4-oz. CWM PP container	freeze at ≤-18°C	6 months to analyze	refrigerate at 4°C	14 days to analyze
Mercury (CVAA)	4-oz. CWM PP container	freeze at ≤-18°C	28 days to analyze	refrigerate at 4°C	28 days to analyze
Total metals (ICP-MS)	4-oz. CWM PP container	freeze at ≤-18 °C	2 years to analyze	refrigerate at 4°C	180 days to analyze
PCB Homologs	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
Lipids	16-oz. glass	freeze at ≤-18°C	1 year to extract Analyze immediately after extraction	refrigerate at 4°C	14 days to extract Analyze immediately after extraction
PBDEs	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
PAHs	8-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
Stable Isotopes	8-oz. glass	freeze at ≤-18°C	indefinitely	refrigerate at 4°C	indefinitely

CWM PP – Clear, wide-mouth polypropylene

4.5 Chain of Custody

Chain of custody (COC) will commence at the time that each crab pot catch is collected. All samples will be under direct possession and control of King County field staff. All sample information will be recorded on a COC form which is the same as the field record sheet (Appendix A) with a KCEL stamp. This form will be completed in the field and will accompany all samples during transport and delivery to the laboratory. Upon arrival at the KCEL, the samples will be relinquished to sample login. The date and time of sample delivery will be recorded and both parties will then sign off in the appropriate sections on the COC form at this time. Once completed, original COC forms will be archived in the project file.

4.6 Field replicates

Field replicates are intended to show within station variability such as may exist at a sediment or water sampling station. For tissue samples, a true field replicate can't be collected because each organism at a station is inherently different (e.g., age, size, diet) so no individuals would be expected to have exactly the same tissue chemistry. Collection and analysis of trip blanks or equipment blanks from crabbing equipment is neither practical nor necessary for this project. The amount of contamination from equipment or sample exposure during transport to the laboratory is relatively insignificant compared to the concentrations measured in tissue.

5.0. SAMPLE DOCUMENTATION

Sampling information and sample metadata will be documented using the methods noted below.

- Field sheets and field observation forms will be used at all stations and will include the following information:
  1. station name (locator)
  2. crab species collected
  3. coordinates of each crab pot per deployment, crab pot number, and counts of how many of each of the targeted species were retained from each pot deployment
  4. date and time of sample collection
  5. counts of species and approximate size of each returned to the water
  6. notes on condition of crab retained
  7. deviations from sampling procedures
  8. unusual conditions (e.g., water color or turbidity, presence of oil sheen, odors)
  9. names of all sampling personnel

- Crab processing sheets will be completed recording station name (locator), pot number, species, lengths, and weights of individuals, and sex of individuals. Sample compositing sheets will be completed recording station name (locator), species, tissue type, LIMS Sample ID, and sample mass aliquot, and number of individuals from which the composite was derived (see Appendix C for details).
- LIMS-generated container labels will identify each container with a unique sample number, station and site names, collect date, analyses required, and preservation method.
- COC documentation will consist of the KCEL standard COC stamp on the field record sheet (Appendix A), which is used to track release and receipt of each sample from collection to arrival at the lab.

6.0. ANALYTICAL PARMETERS AND METHODS

Analytical methods and associated detection limit goals and quality control requirements for the analyses performed are presented in this section. The detection limit goals for metals, total mercury, PCBs, PBDEs and chlorinated pesticides have been updated based on changes to the SW-846 solids methods. The terms "method detection limit" or MDL and "reporting detection limit" or RDL are no longer being used to define sensitivity for these parameters. The EPA analytical reference methods used for this project belong mostly to the SW-846 compendium of analytical methods and require that sensitivity be defined as the "lower limit of quantitation" (LLOQ).

The LLOQ can be no lower than the lowest concentration on the calibration curve and must be verified according to the requirements in each reference method. For SW-846 methods, concentrations less than the LLOQ will be qualified in the KCEL LIMS with a "<QL" flag. LLOQs for all parameters except metals are calculated on a wet-weight (ww) basis using 15 g of sample to 0.5 mL final volume, not corrected for lipid content or total solids. LLOQs for metals are based on an initial sample weight of 1.25 (+/- 0.05) g and a final volume of 50 ml for ICP-MS metals. The listed LLOQs can change in any given sample due to matrix interference, sample dilutions and/or sample weight extracted. LLOQs are periodically evaluated and may increase or decrease. Every effort will be made to meet these ww quantitation limit goals. Any changes to the instrument LLOQ values will be documented in a Data Anomaly Form or project narrative as a deviation from the SAP.

For metals (Methods 6020B and 7471B), PCBs as Aroclors (Method 8082A) and Chlorinated Pesticides (Method 8081B), sample results may not be reported below the LLOQ and, therefore, the LLOQ value for each parameter is entered into both MDL and RDL fields in LIMS.

Sample results for PCB Homologs and PBDE Congeners may be reported at concentrations below the LLOQ if the qualitative results for the parameter meet the requirements of the reference method. In LIMS, the LLOQs will be reported in the LIMS RDL field, while the MDL will represent the minimum concentration where the qualitative criteria for the method can be met (typically one-half the LLOQ). Numeric values reported between the LLOQ and the limit of qualitative method criteria will be flagged "<QL, J", since they are considered estimates.

For analyses not based on SW-846 reference methods such as percent lipids and solids, the sensitivity limit is still defined as the MDL, which is calculated using the procedure in 40 CFR Part 136, Appendix B. The value determined by this procedure may be increased to account for method variability, and will be reported. The RDL is calculated by multiplying the MDL by a factor between 2 and 10, depending on the parameter, and should be considered analogous to a Practical Quantitation Limit (PQL). For methods where the MDL is applied, parameters less than the MDL will be qualified with a "<MDL" flag.

Actual KCEL LLOQs, MDLs, and RDLs may differ from the target detection limit goals as a result of necessary analytical dilutions or a reduction of extracted sample amounts based

upon available sample volumes. Every effort will be made to meet the detection limit goals listed in the SAP.

6.1 Total Metals

Metals samples will be analyzed by inductively coupled plasma mass spectrometer (ICP-MS) according to KCEL SOP # 623. This method generally follows PSEP (1997) and EPA SW-846 6020A protocols. This SW-846 method does not allow data to be reported below the LLOQ. Tissue samples require acid digestion before analysis. LLOQs for ICP-MS metals are based on an initial sample weight of 1.25 (± 0.05) g and a final volume of 50 ml. Detection limit goals for the fourteen target trace metals are summarized in Table 5.

Table 5. Metals target analytes and detection limit goals (mg/Kg ww)

Analyte	LLOQ*
Silver	0.0016
Arsenic	0.004
Barium	0.02
Cadmium	0.002
Chromium	0.008
Copper	0.008
Molybdenum	0.004
Nickel	0.004
Lead	0.004
Antimony	0.012
Selenium	0.02
Thallium	0.004
Vanadium	0.003
Zinc	0.02

* LLOQ = Lower Limit of Quantitation.
Method blanks will be evaluated down to a value that is one-half the LLOQ.

6.2 Total Mercury

Total mercury will be analyzed according to KCEL SOP # 604 using cold vapor atomic absorption spectrometry (CVAA). The analysis is reported under PSEP (1997), which retains method elements of EPA 245.1 revision 3, SW-846 7470 and 7471B. Tissue samples require acid digestion before analysis. LLOQs for mercury are based on an initial sample weight of 0.667 (±0.05) g and a final volume of 100 ml. The detection limits targeted, which are calculated on a ww basis, are shown in Table 6.

Table 6. Mercury detection limit goals (mg/Kg ww)

Analyte / Range	LLOQ*
Mercury (Low Range)	0.00038
Mercury (Mid-Range)	0.004

6.3 Total PCBs

PCB homolog analysis will follow KCEL draft SOP #782, which generally follows the guidelines of EPA methods 680 and 1668C. This method relies on the quantitation of congeners using gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM). This low-resolution method will generate PCB concentrations based on each of the 10 homolog groups. Sample preparation is described in KCEL SOP# 705 for soils, tissues, and sediments. The preparation method is a soxhlet technique following EPA method SW-846-3540C using methylene chloride as the extraction solvent. The Percent Lipids are co-extracted with the PCBs and split 50/50 prior to cleanup. The cleanup method is based on Method 1668C with gel permeation cleanup (GPC) followed by an anthropogenic column clean up, KCEL SOP #718 and #783. The PCB homologs are listed with their associated LLOQs for tissue samples in Table 7. LLOQs for PCB homologs are based on an initial sample weight of 20 (±0.05) g and a final volume of 1.0 ml. SW-846 method allows reporting of values below LLOQ if confirmed by mass spectrometry. These values would be considered estimated values and J qualified.

Table 7. PCB homolog detection limit goals (µg/kg ww)

Homolog group	LLOQ
Monochlorobiphenyls	0.125
Dichlorobiphenyls	0.125
Trichlorobiphenyls	0.125
Tetrachlorobiphenyls	0.25
Pentachlorobiphenyls	0.25
Hexachlorobiphenyls	0.25
Heptachlorobiphenyls	0.375
Octachlorobiphenyls	0.375
Nonachlorobiphenyls	0.625
Decachlorobiphenyl	0.625

*LLOQ = Lower Limit of Quantitation.

6.4 PBDE Congeners

PBDE congener analysis will follow KCEL SOP #781. The method relies on the quantitation of congeners using gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI). Fourteen most commonly detected congeners are quantified using this method. Sample preparation is described in SOP# 705 for soils, tissues, and sediments. The preparation method is a soxhlet technique following EPA method SW-846-3540C using methylene chloride as the extraction solvent. The extract will have GPC, TBA, Alumina, and Acid cleanups performed per KCEL SOP #718, 721, 719, and 720 respectively. After cleanups the extract will be vialed at 1.0 mL for PBDEs. The PBDEs are listed with their associated detection limit goals for tissue samples in Table 8. LLOQs for PBDEs are based on an initial sample weight of 10 (±0.05) g and a final volume of 1.0 ml. SW-846 method allows reporting of values below LLOQ if confirmed by mass spectrometry. These values would be considered estimated values and J qualified.

Table 8. PBDE Congener Detection Limit Goals in (µg/kg ww)

BDE congener	Congener #	LLOQ*
2,2',4-TriBDE	17	0.04
2,4,4'-TriBDE	28/33	0.04
2,2',4,4'-TetraBDE	47	0.36
2,3',4,4'-TetraBDE	66	0.058
2,3',4',6-TetraBDE	71	0.04
2,2'3,4,4'-PentaBDE	85	0.04
2,2'4,4'5-PentaBDE	99	0.68
2,2',4,4'6-PentaBDE	100	0..112
2,2',3,4,4',5' HexaBDE	138	0.04
2,2',4,4',5,5'-HexaBDE	153	0.04
2,2',4,4',5',6-HexaBDE	154	0.044
2,2',3,4,4',5',6-HeptaBDE	183	0.04
2,3,3',4,4',5,6-HeptaBDE	190	0.04
2,2',3,3',4,4',5,5',6,6'-DecaBDE	209	0.5

*LLOQ = Lower Limit of Quantitation.

6.5 PAHs

PAHs analysis will follow KCEL SOP #731v6. The method relies on the quantitation of PAH compounds using gas chromatography/mass spectrometry- selected ion monitoring (GC/MS-SIM). Sample preparation is described in SOP# 705 for soils, tissues, and sediments. The preparation method is a soxhlet technique following EPA method SW-846-3540C using methylene chloride as the extraction solvent. The sample will have GPC performed per KCEL SOP #718. After cleanups the extract will be vialed at 1.0 mL for PAHs. The PAHs are listed with their associated detection limit goals for tissue samples in Table 9. LLOQs for PAHs are based on an initial sample weight of 10 (±0.05) g and a final volume of 1.0 ml. SW-846 method allows reporting of values below LLOQ if confirmed by mass spectrometry. These values would be considered estimated values and J qualified.

Table 9. PAH compounds and associated detection limit goals (µg/kg ww)

PAH	LLOQ
Benzo(a)anthracene	10
Benzo(a)pyrene	10
Benzo[g,h,i]perylene	10
total benzo-fluoranthenes	30
Chrysene	10
Dibenzo(a,h)anthracene	10
Ideno(1,2,3-cd)pyrene	10
Fluoranthene	10
Pyrene	10
Acenaphthylene	5
Acenaphthene	5
Anthracene	10
Fluorene	10
Naphthalene	5
Phenanthrene	10
2-Methylnaphthalene	5

*LLOQ = Lower Limit of Quantitation.

6.6 Other Parameters

Total solids will be analyzed according to SM2540-G. The total solids analysis will follow KCEL SOP # 307. The MDL is 0.005 % and the RDL is 0.010 % for tissue samples. Lipid analysis will be conducted following KCEL draft SOP # 740. Samples are extracted by the same method as the PCBs. The lipid analysis MDL and RDL are 0.05 and 0.1 %, respectively.

Carbon and nitrogen stable isotopes (Solid $\delta^{13}C$, $\delta^{15}N$, respectively) will be analyzed by isotope ratio mass spectrometer (IsoLab 2017). There are no cleanup or extraction methods associated with this analysis. The MDLs are 0.1 per ml for $\delta^{13}C$ and 0.2 per ml for $\delta^{15}N$. The analysis is expected to be performed by University of Washington IsoLab.

7.0. LABORATORY QUALITY CONTROL

This section presents laboratory quality assurance/quality control (QA/QC) measures that will be employed to ensure data are of sufficient quality to meet the project DQOs.

An analytical batch is defined as a maximum of 20 analytical samples plus QC samples. QC samples can include method blanks, spiked blanks, matrix spikes, matrix spike duplicates, lab duplicate, lab triplicates, lab control samples, lab control sample duplicates, SRMs and SRMDs (when available). For organic samples, surrogates are also added to every sample. QC samples will be analyzed at the frequency of one per QC batch, defined as up to 20 samples analyzed together. Standard Reference Materials (SRMs) will be used whenever available. Some analyses have empirically derived laboratory limits for various QC samples; those in place at the time of sample analysis will be followed. These will be included in the laboratory QC reports.

QC results that exceed the acceptance limits will be evaluated to determine appropriate corrective actions. Samples will typically be reanalyzed if the unacceptable QC results indicate a systematic problem with the overall analysis. Unacceptable QC results caused by a particular sample or matrix will not require reanalysis unless an allowed method modification would improve the results. The laboratory QC sample requirements for each analysis are summarized in Table 10. The recommended QC limits for tissue analysis are in Table 11.

Table 10. Minimum quality control samples by analysis

Parameter	Blank[1]	Duplicate[2]	Matrix Spike	LCS and/ or SRM[3]	Surrogates	Spiked Blank
Metals	1 Per Batch	1 Per Batch	1 Per Batch	Yes	No	1 Per Batch
Mercury	1 Per Batch	1 Per Batch[4]	1 Per Batch	Yes	No	1 Per Batch
PCB Homologs	1 Per Batch	No	1 Per Batch[5] (MS/MSD)	No	Yes[6]	1 Per Batch
PBDEs	1 Per Batch	No	1 Per Batch[5] (MS/MSD)	Yes	Yes	1 Per Batch
PAHs	1 Per Batch	No	1 Per Batch[5] (MS/MSD)	No	Yes	1 Per Batch
Total Solids	1 Per Batch	1 Per Batch	No	No	No	No
Lipids	1 Per Batch	1 Per Batch	No	No	No	No
Stable isotopes	1 Per Batch	1 per batch	No	Yes	No	No

[1] Batch - A group of samples analyzed together for QC purposes containing a maximum of 20 samples, except for stable isotopes where batch is 30 samples.

[2] Duplicate - Triplicate analysis for all conventional parameters, duplicate analysis for metal parameter.

[3] SRM - Standard reference material (must be certified by NIST or NRCC). For stable isotopes, GA1, GA2 and salmon will be used to assess accuracy. LCS - Lab Control Sample.

[4] Mercury samples are analyzed with a Matrix Spike and Matrix Spike Duplicate.

[5] MS/MSD analyzed if sample volume allows, otherwise a spiked blank duplicate will be analyzed.

[6] Decachlorobiphenyl is a target analyte for PCB homologs

Table 11. Quality control limits for tissue samples.

Parameter	Blank[1]	Replicate[2]	Matrix Spike[3]	SRM[4]	Surrogates	Spiked Blank
Metals	< ½ LLOQ	≤ 20%	75 - 125%	varies	N/A	85 to 115%
Mercury	< ½ LLOQ	≤ 20%	75 - 125%	80 to 120%	N/A	85 to 115%
PCB Homologs	< ½ LLOQ	≤ 35%	varies	N/A	varies	varies
PBDEs	< ½ LLOQ	≤ 40%	varies	50 to 150%	varies	50 to 150%
PAHs	< ½ LLOQ	≤ 35%	varies	N/A	varies	varies
Lipids	<MDL	≤ 20%	N/A	N/A	N/A	N/A
Total Solids	< MDL	≤ 20%	N/A	N/A	N/A	N/A
Stable Isotopes	<MDL	TBD	N/A	± 0.1 (13C) and 0.2 (15N)	N/A	N/A

NOTES:

[1]For SW-846 methods, the comparison for blanks is ½ the ww LLOQ value. For non-SW-846 methods, the concentrations in blanks shall be below the ww MDL.

[2]Relative percent difference (RPD) for duplicate analysis and percent relative standard deviation (%RSD) for triplicate analysis; triplicate analyses applies to total solids and lipids.

[3]Percent recovery for matrix spike, standard reference material, and surrogates.

[4]If SRM or LCS is available.

N/A – not applicable

8.0. DATA ASSESSMENT, REPORTING AND RECORD KEEPING

This section presents how data related to this study will be reported and stored.

8.1 Data Assessment

Data assessment is critical for evaluating how well analytical data meet project DQOs. Data assessment is performed, at some level, during several steps in the process of sample analysis. Data assessment will also be performed by the KCEL QA Officer or Lab Project Manager for this program by reviewing complete data packages supplied by the KCEL. Data assessment memoranda will be produced and maintained along with the analytical data as part of the project records.

8.2 Reporting

Data will be reported in a technical memorandum or report that will include a presentation and interpretation of the crab tissue data results. In addition, the memorandum or report will detail any anomalies seen between this dataset and historical data available. Due to natural variability (spatially, temporality, and between individuals) and the lack of sample replicates (particularly in historical datasets), it is not expected that enough statistical power will be available to test for significance differences. The narrative will include summary data tables, descriptions of how the data were generated, including sampling and analysis descriptions. Data packages and the QA review will be included as appendices.

8.3 Record Keeping

All hard-copy field sampling records, custody documents, raw lab data, and laboratory summaries and narratives will be archived according to KCEL policy for a minimum of 10 years from the date samples were collected. A copy of the technical memorandum will be maintained in the DNRP Library.

9.0. REFERENCES

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Appendix A
Field Record Sheet

Field Record for 2017 West Point Incident Crab Tissue Monitoring Event

Date of Collection: _____

Approximate Time: _____

Location: _____

Equipment: _____

Pot Number: _____

Pot Coordinates: _____

_____

Species[1]	# Taken	Returned		Observations[2]
		Number	Approximate size ranges	

[1] To lowest taxonomic level possible.

[2] Includes general disposition of species

Notes:_____

_____

_____

_____

Field Personnel: _____

Appendix B

May Sample Event Collection Memo

MEMORANDUM

To: Ben Budka, KC Environmental Lab
Bob Kruger, KC Environmental Lab
Fritz Grothkopp, KC Environmental Lab

From: Debra Williston, Toxicology and Contaminant Assessment Unit, WLRD

Cc: Jeff Lafer, Environmental & Community Services, WTD
Bruce Nairn, Modeling/GIS, WTD
Deb Lester, Toxicology and Contaminant Assessment Unit, WLRD
Jenee Colton, Toxicology and Contaminant Assessment Unit, WLRD

Subject: Dungeness crab collection related to West Point Treatment Plan Incident

Date: April 19, 2017

The memo outlines the crab sampling design for tissue sampling supporting the West Point Treatment Plant incident. Dungeness crab will be sampled this year to look for gross anomalies and support evaluation of long-term tissue monitoring data. This collection effort is a collaboration between King County and Washington Department of Fish and Wildlife.

The sampling and crab processing procedures outlined in the *2014 Elliott Bay and Puget Sound Crab Tissue Sampling and Analysis Plan* (SAP) (King County 2014) will be followed except as outlined in this memo. The field sheets from this SAP will also be used. The analytical requirements have not been finalized, and thus, a separate memo will be developed after collection efforts are completed and the analyte list is confirmed.

Two sampling events will be conducted. Samples will first be collected in April 2017 while the West Point Treatment plant is not yet running at full treatment capacity (secondary treatment). Samples will also be collected after the treatment plant is running at full treatment capacity. This is expected to occur during summer (the specific timing will be communicated in an email).

Samples will be collected offshore from two locations at Shilshole Bay Marina: Shilshole Bay Marina North and South (see below). Two to three crab pots will be deployed within approximately 200-400 yards of the respective piers.

Sampling Location	Locator ID	General Location Coordinates[a]	
		X	Y
Shilshole North	CB-SHMarina-N	1253476	254630
Shilshole South	CB-SHMarina-S	1251797	250856

[a] Coordinates represent general sampling locations. Coordinates are in State Plane North NAD83 US Survey Feet.

The target numbers of male Dungeness crab for each sampling location are shown below. A total of 30 crabs from the two locations are targeted (15 crabs per location). Crab pot deployment will occur 2 to 3 nights depending on the success of the sampling the previous day(s).

Species	Tissue type	Target number of composites	Target number of crab per composite	Total Crab per Location
Dungeness Crab	Muscle	5	3	Up to 15
Dungeness Crab	Hepatopancreas	3	5	Dissected from whole

The crab will be collected in April under an authorization memo from WDFW. WDFW may have a staff person on the boat to assist with the collection effort. The crab pots will need to be tagged with the WDFW's name/address. As previous collection effort, the field sheets will need to track all species collected and released from the traps and number of Dungeness male crab retained. See Appendix A of Crab SAP for field collection/chain of custody forms. For the second field effort this summer, we expect to obtain a King County collection permit from WDFW, otherwise we will follow the same procedures for April effort.

Crab processing following collection will include length and width measurements using the appropriate field processing sheets (see Appendix C of crab SAP). Crab will then be stored frozen until compositing scheme is confirmed (based on number of crabs collected) and analyte list is confirmed.

King County. 2014. 2014 Elliott Bay and Puget Sound Crab Tissue Monitoring Sampling and Analysis Plan. Prepared by Richard Jack and Debra Williston, Science and Technical Support Section, King County Water and Land Resources Division. Seattle, Washington

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Appendix C

Sample Processing and Compositing Sheets

Sample Processing for 2017 West Point Incident Crab Tissue Monitoring Event

Date of Collection: _____

Locator: _____

Species: _____

Pot Number: _____

Individual # (sequential)	Total Carapace Length (mm)	Sex	Whole Body Mass (g)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
	--	--	

Notes:_____
_____
_____

Lab Personnel:_____

Sample Compositing Sheet for 2017 West Point Incident Crab Tissue Monitoring

Date of Collection(s): _____

Locator: _____

Species: _____

Tissue Type Muscle / Hepatopancreas

Sample ID: _____

Crab Pot #	Individual # (sequential)[1]	Total Carapace Length (mm)	Sex	Mass (g) Aliquot
Totals		--	--	

[1] From sample processing sheet.

Notes:_____
_____
_____

Lab Personnel:_____